PB84-149715

# Comparison of Nitro-Aromatic Content and Direct-Acting Mutagenicity of Passenger Car Engine Emissions

Battelle Columbus Labs., OH

Prepared for

Health Effects Research Lab.
Research Triangle Park, NC

Jan 84

U.S. Department of Commerce
National Technical Information Service
NTIS

PB84-149715

EPA-600/D-84-044
January 1984

# COMPARISON OF NITRO-AROMATIC CONTENT AND DIRECT-ACTING MUTAGENICITY OF PASSENGER CAR ENGINE EMISSIONS

by

M. G. Nishioka and B. Petersen
Battelle Columbus Laboratories
Columbus, OH 43201

and

Joellen Lewtas
Health Effects Research Laboratory
Research Triangle Park, NC 27711

HEALTH EFFECTS RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
RESEARCH TRIANGLE PARK, NC 27711

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

## TECHNICAL REPORT DATA
*(Please read Instructions on the reverse before completing)*

| 1. REPORT NO. | 2. | 3. RECIPIENT'S ACCESSION NO. |
|---|---|---|
| EPA-600/D-84-044 | | PB84 149715 |

| 4. TITLE AND SUBTITLE | 5. REPORT DATE |
|---|---|
| Comparison of Nitro-Aromatic Content and Direct-Acting Mutagenicity of Passenger Car Engine Emissions | January 1984 |
| | 6. PERFORMING ORGANIZATION CODE |
| 7. AUTHOR(S) Marcia G. Nishioka, Bruce Petersen (Battelle), and Joellen Lewtas (USEPA) | 8. PERFORMING ORGANIZATION REPORT NO. |
| 9. PERFORMING ORGANIZATION NAME AND ADDRESS<br>Battelle Columbus Laboratories<br>505 King Avenue<br>Columbus, Ohio 43201 | 10. PROGRAM ELEMENT NO.<br>A9XA1C |
| | 11. CONTRACT/GRANT NO. |
| 12. SPONSORING AGENCY NAME AND ADDRESS<br>Environmental Protection Agency<br>Health Effects Research Laboratory<br>Genetic Toxicology Division, Genetic Bioassay Branch (MD 68)<br>Research Triangle Park, NC 27711 | 13. TYPE OF REPORT AND PERIOD COVERED |
| | 14. SPONSORING AGENCY CODE<br>EPA-600/11 |

15. SUPPLEMENTARY NOTES

16. ABSTRACT

This paper describes a study to identify and quantify 23 nitroaromatic compounds in the extracts of particulate material from three light-duty passenger car diesel engines and one gasoline engine. Mutagenic assay data were also collected on these extracts using the Salmonella typhimurium TA98 bioassay without S9 metabolic activation. The results of these two studies were compared to determine whether the concentration of the nitro-aromatics detected can fully account for the direct-acting mutagenic activity indicated by the bioassay data.

17. KEY WORDS AND DOCUMENT ANALYSIS

| a. DESCRIPTORS | b. IDENTIFIERS/OPEN ENDED TERMS | c. COSATI Field/Group |
|---|---|---|
| | | |

| 18. DISTRIBUTION STATEMENT | 19. SECURITY CLASS *(This Report)* | 21. NO. OF PAGES |
|---|---|---|
| Release to Public | Unclassified | 16 |
| | 20. SECURITY CLASS *(This page)* Unclassified | 22. PRICE |

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

# COMPARISON OF NITRO-AROMATIC CONTENT AND DIRECT-ACTING MUTAGENICITY OF PASSENGER CAR ENGINE EMISSIONS

Marcia G. Nishioka, Bruce Petersen, Joellen Lewtas

Battelle, Columbus Laboratories
U.S. Environmental Protection Agency

Particulate emissions from internal combustion engines are a very complex mixture of carbonaceous matter containing absorbed and/or condensed organic components from the combustion of fuel and lubricants. The organic solvent extractable fraction of these particulates is extremely complex and has been reported to contain hundreds of individual compounds. A number of studies have reported that these solvent extracts are mutagenic as determined by the Ames Salmonella microbial assay. The greatest activity which has been found in certain compound class fractions within these extracts does not require metabolic activation and is called direct mutagenic activity. Chemical characterization studies have been used in an attempt to identify the compound classes or specific compounds present in these fractions. Polynuclear aromatic hydrocarbons (PAH), particularly pyrene, have been reported to readily react with nitrogen oxides to form nitrated derivatives which are powerful direct-acting mutagens. Both the PAHs and nitrogen oxides are present in engine emissions and thus the formation of nitroaromatics may indeed be possible. These results have prompted increased attention to the characterization of particulate extracts to identify the compounds or compound classes which are responsible for the direct acting biological activity. Identification of specific mutagens or classes of mutagens is important to determine whether these components are present in the effluent which is emitted to the atmosphere or are formed as a result of the particulate collection and analysis procedures. This information must be established before potential effects of these compounds on the environment can be addressed.

This presentation will describe a study to identify and

quantify 23 nitroaromatic compounds in the extracts of particulate material from three light-duty passenger car diesel engines and one gasoline engine. Mutagenic assay data were also collected on these extracts using the Salmonella typhimurium TA98 bioassay without S9 metabolic activation. The results of these two studies were compared to determine whether the concentration of the nitroaromatics detected can fully account for the direct-acting mutagenic activity indicated by the bioassay data.

## INTRODUCTION

The increasing number of automobiles and light-duty trucks powered by diesel engines has generated concern over the emissions associated with these engines. Diesel engines have a higher particulate emission rate than that of gasoline catalyst engines. The extractable organics from both diesel and gasoline particle emissions have been found to be mutagenic and carcinogenic[1]. Recently, nitro-substituted polynuclear aromatic hydrocarbons (nitro-PAH, nitro-aromatics) have been identified in diesel particle extracts[2]. Several of these nitro-aromatics are very potent bacterial mutagens[3].

A study was carried out to identify and quantify nitro-aromatic compounds in the extract of particulate material from three different light-duty passenger car diesel engines and one gasoline engine. The operating and sampling conditions have been described elsewhere[4]. Mutagenic assay data were also collected on these extracts using the Salmonella typhimurium TA98 bioassay, without S9 metabolic activation. The results of these two studies were compared to determine whether the concentration of nitro-aromatics detected can fully account for the direct-acting mutagenic activity indicated by the bioassay data.

## TECHNICAL APPROACH

### Chemical

Each particulate extract was separated into four chemical compound classes using open-bed silica gel column chromatography: (1) aliphatic hydrocarbons--hexane elution, (2) polycyclic aromatic hydrocarbons--hexane:benzene (1:1 v/v) elution, (3) moderately polar neutrals--methylene chloride elution, and (4) highly polar neutrals, primarily oxygenated compounds--methanol elution. Fraction #2 was expected to contain the mononitro-aromatics, fraction #3 was expected to contain the di- and trinitro-aromatics, and fraction #4 was expected

to contain the oxygenated nitro-aromatics. The quantity of silica gel and the volume of elution solvent used were adjusted for the amount of total organic material available for fractionation.

A high resolution gas chromatography/negative chemical ionization mass spectrometry (HRGC/NCIMS) method using on-column injection was developed to analyze the particulate extracts. The instrumentation consisted of a Finnigan Model 4000 GC/MS interfaced to a Finnigan/INCOS Model 2300 Data System. Methane was selected as the reagent gas. A J&W DB-5 bonded, fused silica capillary column was used. The on-column injection was made at 40°C, and the GC was temperature programmed from 40-320°C at 10°C/minute. Standard solutions and fractions #2, #3, and #4 of each extract were analyzed by this method.

Standard solutions containing the following nitro-aromatic compounds

| | |
|---|---|
| 1-nitronaphthalene | 2,7-dinitrofluorene |
| 2-nitrofluorene | 2,7-dinitrofluorenone |
| 9-nitroanthracene | 1,6-dinitropyrene |
| 3-nitrofluoranthene | 1,3,6-trinitropyrene |
| 1-nitropyrene | |

were prepared over the concentration range 0.1-10 ng/µl for the calibration curve. Perdeutero ($d_7$) nitronaphthalene was chosen as the internal standard and it was spiked into all standards and samples at the level of 2.5 ng/µl. The response factor for each compound relative to the internal standard was calculated over the calibration range. Nitro-aromatic compounds not present in the standard which were detected in samples were quantified using the response factor of the most similar compound from the standard. In addition, separate solutions of 1,3- and 1,8-dinitropyrene were analyzed to determine the elution order of the three dinitropyrene isomers.

The relative retention time (relative to $d_7$-nitronaphthalene) and mass spectrum of each standard compound were used to assign specific isomer identification to a few of the nitro-aromatics detected in the extracts. The other nitro-aromatic isomers detected were identified by appropriate mass spectra and retention times. The availability of other specific nitro-aromatic isomers may allow assignments to be made of those compounds.

The methanol fractions (#4) of two of the light-duty diesel particulate extracts were also analyzed by HRGC/MS and high resolution mass spectrometry (HRMS) to determine in an attempt to

tentatively identify the formulas of compounds in the most polar class fraction. From these data, structures and compound classes were tentatively assigned.

Biological

An aliquot of the total extract from each sample was tested in severe short-term bioassays as reported previously[1]. The slope of the dose response curve (rev/μg) in the Salmonella typhimurium TA98 plate incorporation assay[5] without metabolic activation (-S9) was utilized in this study to compare the nitro-aromatic content and direct-acting mutagenicity of each sample extract.

RESULTS

Twenty-three different nitro-aromatics were tentatively identified in the three light-duty diesel (LDDI, LDDII and LDDIII) engine extracts. However, only 1-nitropyrene was detected in the gasoline engine extract. In all cases, the 1-nitropyrene was the nitro-aromatic compound detected in greatest quantity, followed by the nitrophenanthrene/anthracene isomers. Quantities of the detected nitro-aromatics are given in Table 1 and vary over the range of 0.1 ppm to 589 ppm in the extracts.

Mono-nitro derivatives of the PAHs at molecular weight 228 (such as benz[a]anthracene and chrysene) and molecular weight 252 (such as benzopyrenes, perylene and benzofluoranthenes) were also detected. Two nitropyrenone isomers were tentatively identified in the LDDI and LDDIII samples and the three dinitropyrene isomers were identified in the LDDII sample.

The chromatograms from the NCI HRGC/MS analysis of the hexane:benzene and methylene chloride fractions of the LDDI diesel extract are shown in Figures 1 and 2, respectively. In spite of the relatively rapid temperature program rate, 10°C/minute, the excellent resolving power of the capillary column was maintained. As shown in Figure 1, analyses were complete in 30 minutes, achieving baseline separation for every significant peak. This resolving power enhanced the accuracy for quantifying closely eluting structural isomers.

In Figure 1, the peaks with intensity greater than 25% of the largest peak were identified as PAHs, with the exception of the peak at scan #1275. This peak was identified as 1-nitropyrene. The methylene chloride fraction was considerably more complex and included primarily carboxaldehyde and semi-quinone derivatives of PAH. The largest peak (scan #850) was identified as 9-fluorenone.

TABLE 1. QUANTIFICATION OF NITRO-AROMATICS DETECTED IN EXTRACTS OF ENGINE PARTICULATE

| Compound | Concentration in Extract, wt ppm | | | | Relative Retention Time[a] |
|---|---|---|---|---|---|
| | LDDI | LDDII | LDDIII | Gasoline | |
| 1-nitronaphthalene | 0.5 | 0.7 | 0.3 | ND | 1.003 |
| Nitronaphthalene/azulene isomer | 0.1 | 0.6 | 0.2 | ND | 1.031 |
| Nitronaphthalene/azulene isomer | 0.9 | 1.2 | 0.3 | ND | 1.042 |
| Nitrofluorene isomer | ND[b] | 1.0 | 0.5 | ND | 1.426 |
| 2-nitrofluorene | ND | ND | 0.4 | ND | 1.478 |
| Nitrofluorene isomer | 1.9 | 1.4 | 1.1 | ND | 1.503 |
| Nitrophenanthrene/anthracene isomer | 98 | 285 | 63 | ND | 1.494 |
| Nitrophenanthrene/anthracene isomer | 2.7 | 33 | 25 | ND | 1.545 |
| 3-nitrofluoranthene | 2.9 | 1.2 | 0.9 | ND | 1.804 |
| Nitrofluoranthene/pyrene isomer | 1.7 | 1.4 | 0.6 | ND | 1.813 |
| 1-nitropyrene | 407 | 589 | 107 | 2.5 | 1.842 |
| Nitromethylpyrene isomer | 23 | 46 | 22 | ND | 1.843 |
| Nitromethylpyrene isomer | 1.9 | 3.3 | 1.9 | ND | 1.874 |
| Nitro 228[c] | 3.0 | 1.1 | 1.4 | ND | 1.881 |
| Nitro 228 | 0.8 | 1.3 | 0.2 | ND | 1.938 |
| Nitro 228 | 3.9 | 5.6 | 1.2 | ND | 1.977 |
| Nitromethyl 228 | 2.6 | ND | ND | ND | 2.132 |

TABLE 1. (CONTINUED)

| Compound | Concentration in Extract, wt ppm | | | | Relative Retention Time [a] |
|---|---|---|---|---|---|
| | LDDI | LDDII | LDDIII | Gasoline | |
| Nitro 252[d] | 29 | 10.0 | 1.1 | ND | 2.232 |
| Nitropyrenone isomer[e] | 4.8 | ND | 2.1 | ND | 1.901 |
| Nitropyrenone isomer[e] | 11.5 | ND | 3.9 | ND | 1.916 |
| 1,3-dinitropyrene | ND | 0.6 | ND | ND | 2.090 |
| 1,6-dinitropyrene | ND | 0.6 | ND | ND | 2.126 |
| 1,8-dinitropyrene | ND | 0.4 | ND | ND | 2.149 |

(a) Relative to internal standard, $d_7$-nitronaphthalene.
(b) Not detected.
(c) PAH isomers of MW 228-benz(a)anthracene, chrysene, benz(c)phenanthrene, etc.
(d) PAH isomers of MW 252-B(e)P, B(a)P, perylene, benzofluoranthenes, etc.
(e) Tentative identification.

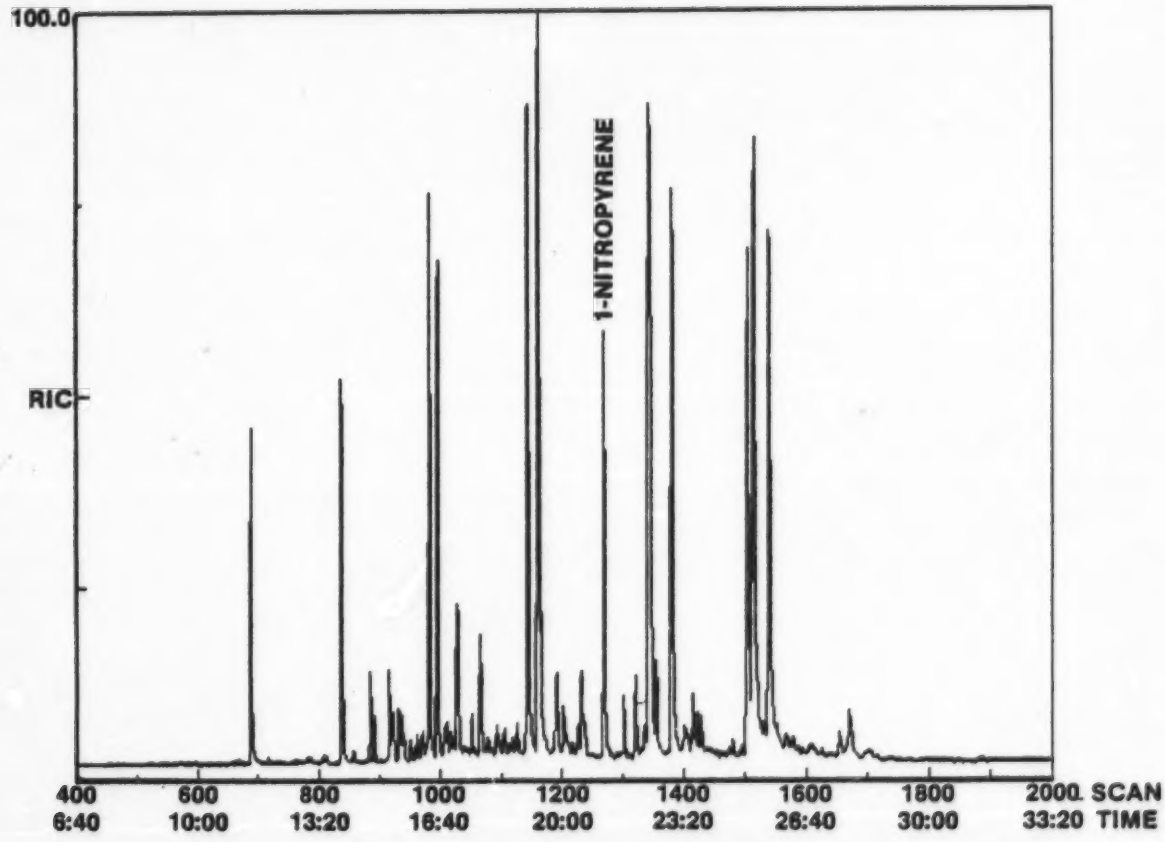


**FIGURE 1. NCI HRGC/MS CHROMATOGRAM OF HEXANE/BENZENE FRACTION OF LDDI PARTICULATE EXTRACT**

The mutagenic activity (rev/μg) by linear regression analysis for the engine emission extracts and selected nitro-aromatic compounds is given in Table 2. Note that the commercially available 1-nitropyrene appears to be contaminated with di-nitropyrenes.

Analyses by high-resolution mass spectrometry and electron impact HRGC/MS were used to determine that most of the compounds in the methanol fraction of the LDDI and LDDII extracts were quinones, ketones, and dicarboxylic acid anhydride derivatives of PAHs. Representative compounds tentatively identified in the methanol fractions are given in Table 3.

TABLE 2. MUTAGENIC ACTIVITY OF ENGINE EXTRACTS AND PURE NITRO-AROMATICS

| Sample | Mutagenic Activity, rev/μg TA98,-S9 | Reference |
|---|---|---|
| LDDI | 12.0 | |
| LDDII | 3.7 | |
| LDDIII | 5.8 | |
| Gasoline | 1.1 | |
| 1-nitronaphthalene | 0.3 | 3 |
| 2-nitronaphthalene | 1.2 | 3 |
| 2-nitrofluorene | 65.9 | 3 |
| 3-nitrofluoranthene | 22,500 | 6 |
| 1-nitropyrene (commercially available) | 2,111 | 7 |
| 1-nitropyrene (99.99% pure) | 918 | 7 |
| 1,3-dinitropyrene | 496,000 | 8 |
| 1,6-dinitropyrene | 629,000 | 8 |
| 1,8-dinitropyrene | 870,000 | 8 |
| nitrophenanthrene (nitration mix) | 240 | 9 |
| nitroanthracene (nitration mix) | 1.8 | 9 |
| nitrochrysene (nitration mix) | 190 | 9 |
| nitroperylene (nitration mix) | 654 | 9 |

TABLE 3. REPRESENTATIVE COMPOUNDS TENTATIVELY IDENTIFIED IN METHANOL FRACTIONS

| Tentatively Identified Compound | Accurate Mass | Measured Mass Assignment Accuracy, ppm | Formula |
|---|---|---|---|
| Fluorenone | 180.0574 | -2.7 | $C_{13}H_8O$ |
| Acenaphthylenedione | 182.0367 | -3.4 | $C_{12}H_6O_2$ |
| Anthracenone | 194.0730 | 0.5 | $C_{14}H_{10}O$ |
| Naphtho-pyrandione | 198.0315 | -0.2 | $C_{12}H_6O_3$ |
| Anthracendione | 208.0522 | -3.7 | $C_{14}H_8O_2$ |
| Pyrenedione | 232.0523 | 0.4 | $C_{16}H_8O_2$ |
| Phenanthrenediquinone | 238.0264 | 6.3 | $C_{14}H_6O_4$ |
| Cyclopenta(def)-chrysenone | 254.0731 | -1.1 | $C_{19}H_{10}O$ |
| Benzo(ghi)fluor-anthenedione | 256.0522 | 6.7 | $C_{18}H_8O_2$ |
| Pyrene-pyrandione | 272.0471 | 3.8 | $C_{18}H_8O_3$ |

## CONCLUSIONS

Negative chemical ionization high-resolution gas chromatography/mass spectrometry with on-column injection (NCI HRGC/MS) provides sensitivity and selectivity for the detection of nitro-aromatics. The limit of detection is approximately 50 pg for the mono-nitro-aromatics and di-nitro-pyrenes in the full mass scan data acquisition mode. The limit of detection can be improved, if necessary by using selected ion monitoring techniques.

The NCI on-column injection HRGC/MS method provides significant benefits for the analysis of nitro-aromatics over the conventional EI GC/MS methods. It provides for injection into the chromatographic system at a lower temperature in order to avoid degradation of the thermally labile nitro-aromatics. The chemical ionization technique is less energetic than electron impact and thus enhances sensitivity for detection of a molecular ion by decreasing the amount of fragmentation. The negative chemical ionization technique is especially sensitive and selective for the detection of nitro-aromatics. This is due to the electro-negative nature of the nitro substitution which is highly susceptible to attachment of a thermal electron from the reagent gas plasma. Theoretically, the greater the nitro substitution the higher the electronegativity and thus the lower the limit of detection. This enhanced sensitivity has been observed only when an extremely well deactivated GC column was used. Column active sites presumably cause the irreversible adsorption of nitro-aromatics and the magnitude of the effect is greater for the di- and tri-nitro compounds than for the mono-nitro compounds. In practice, sensitivity for mono- and di-nitro compounds is approximately the same. For the reasons listed above, the NCI on-column injection HRGC/MS method is the most sensitive and selective method identified to date for the analysis of nitro-aromatics.

For each extract, the percentage of measured mutagenic activity due to 1-nitropyrene was calculated and is given in Table 4. The value of 918 rev/μg was used as the mutagenic activity of 1-nitropyrene for these calculations. In addition, the percentage of measured mutagenic activity due to those nitro-aromatics detected by mass spectrometry was calculated for each extract using the specific activities listed in Table 2.

The nitration mixes produced synthetically were assumed to be similar in isomer distribution to that found in the diesel extracts. The mutagenic activity of each nitration mix was applied to the appropriate isomers detected in the extracts.

Since the calculated contribution to mutagenic activity from identified nitro-aromatics is well below 100 percent, several conclusions should be considered: (1) nitro-aromatics whose activities are unknown to date contribute significantly to the direct acting activity, (2) compounds other than nitro-aromatics, present in the extracts, are potent mutagens, or (3) nitro-aromatics originally present in the extract oxidized or decomposed with time.

The identification of compounds present in the methanol fractions and the detection of the two nitropyrenone isomers tend to support the hypothesis of oxidation occurring in the

TABLE 4. PERCENTAGE CONTRIBUTION OF NITRO-AROMATICS TO DIRECT-ACTING MUTAGENIC ACTIVITY OF EXTRACTS

| Sample | Measured Mutagenic Activity[a] rev/μg | Calculated Percentage Contribution to Mutagenic Activity due to: 1-Nitro-Pyrene | Identified Nitro-Aromatics |
|---|---|---|---|
| LDDI | 12.0 | 3.1 | 4.3 |
| LDDII | 3.7 | 14.6 | 37.5 |
| LDDIII | 5.8 | 1.7 | 2.9 |
| Gasoline | 1.1 | 0.2 | 0.2 |

a = TA98, -S9 assay, Reference 5

diesel engine extracts during storage. This hypothesis is further supported by data from bioassays of the LDDI extract which had been stored for two years. These recent bioassays indicated much reduced mutagenic activity and higher toxicity for the stored extract than measured originally in the fresh extract. Additionally, quinones and keto-aromatics are generally toxic but not mutagenic. It is possible that the nitro-aromatics may have originally accounted for a larger percentage of the activity, but with storage, many of the nitro-aromatics may have been oxidized to quinones and keto-aromatics.

These observations may indicate that a greater concentration of nitro-aromatics might have been originally present in the extracts. This possibility was investigated by extracting a particulate sample from LDDI which had been stored for two years. Both a bioassay and analysis for nitro-aromatics were performed on this freshly prepared extract. The mutagenic activity was found to be 12.8 rev/μg and is virtually the same results as was determined two years ago. Analytical results are presented in Table 5 along with the results from the analysis of the stored extract. Differences in the nitroaromatic concentrations between the freshly extracted particulate and the stored extract demonstrate that the nitroaromatics degrade substantially on storage. It is important to note that the 1-nitropyrene concentration is about a factor of four higher in the freshly

prepared extract that in the stored extract (1587 ppm vs 407 ppm). Its proportion to the mutagenic activity of the extract thus increases from 3.1 percent to 12.1 percent. These results indicate that the nitroaromatics may be more stable when adsorbed or condensed onto the soot particles than in a concentrated organic solvent extract. Biological and chemical characterization of particulates is very ddpendent on sample preparation and therefore must be conducted simultaneously to assure that the sample integrity is maintained. Standardization of methods for analysis of nitroaromatics is currently under way.

TABLE 5. CONCENTRATION OF NITRO-AROMATICS IN LDDI PARTICULATE EXTRACTS

| Compound | Concentration in Extract, wt ppm | |
|---|---|---|
| | Freshly Extracted Stored Particulate | Stored Extract |
| 1-nitronaphthalene | 1.4 | 0.5 |
| Nitrofluorene isomer | 18 | 1.9 |
| Nitrophenanthrene | 272 | 98 |
| Nitroanthracene | 8.8 | 2.7 |
| 3-nitrofluoranthene | 7.0 | 2.9 |
| Nitrofluoranthene/pyrene isomer | 7.5 | 1.7 |
| 1-nitropyrene | 1,587 | 407 |
| Nitromethylpyrene isomer | 233 | 23 |
| Nitromethylpyrene isomer | 11 | 1.9 |
| Nitrated 228 MW PAH | 16 | 3 |
| Nitrated 228 MW PAH | 7.2 | 0.8 |
| Nitrated 228 MW PAH | 31 | 3.9 |
| Nitrated 242 MW PAH | 2.8 | 2.6 |
| Nitrated 252 MW PAH | 0.4 | ND(a) |
| Nitrated 252 MW PAH | 1.2 | ND |
| Nitrated 252 MW PAH | 68 | 29 |
| Nitropyrenone isomer(b) | 15 | 4.8 |
| Nitropyrenone isomer | 35 | 12 |

(a) Not detected
(b) Tentative identification

REFERENCES


1. Nesnow, S., and Huisingh, J. L. Mutagenic and carcinogenic potency of extracts of diesel and related environmental emissions: Summary and discussion of the results. In: Health Effects of Diesel Engine Emissions, Vol. II. Edited by W. E. Pepelko, R. M. Danner, and N. A. Clarke, EPA-600/9-80-057b, U.S. Environmental Protection Agency, Cincinnati, Ohio, 1980, pp. 898-912.

2. Petersen, B. A., Chuang, C., Margard, W., and Trayser, D. Identification of mutagenic compounds in extracts of diesel exhaust particulates. In: Proceedings of the 74th Annual APCA Meeting, Philadelphia, Pennsylvania, 1981.

3. Rosenkranz, H. S., McCoy, E. C., Sanders, D. R., Butler, M., Kiriazides, D. K., and Mermelstein, R., 1980. Nitropyrenes: Isolation, identification and reduction of mutagenic impurities in carbon black and toners. Science 209:1038-1043.

4. Huisingh, J. L., Bradow, R. L., Jungers, R. H., Harris, R. D., Zweidinger, R. B., Cushing, K. M., Gill, B. E., and Albert, R. E. Mutagenic and carcinogenic potency of extracts of diesel and related environmehtal emissions: Study design, sample generation, collection, and preparation. In: Health Effects of Diesel Engine Emissions, Vol. II, Edited by W. E. Pepelko, R. M. Danner, and N. A. Clarke, EPA-600/9-80-057b, U.S. Environmental Protection Agency, Cincinnati, Ohio, 1980, pp. 788-800.

5. Claxton, L. D. Mutagenic and carcinogenic potency of diesel related environmental emissions: Salmonella bioassay. In: Health Effects of Diesel Engine Emissions, Vol. II, Edited by W. E. Pepelko, R. M. Danner, and N. A. Clarke, EPA-600/9-80-057b, U.S. Environmental Protection Agency, Cincinnati, Ohio, 1980, pp. 801-807.

6. Austin, A., and Lewtas, J., private communication.

7. Austin, A., and Lewtas, J., private communication.

8. Mermelstein, R., Rosenkrantz, H. S., and McCoy, E. C., Microbial Mutagenicity of Nitroarenes, In Genetoxic Effects of Airborne Agents. Edited by R. Tice, D. L. Costa and K. M. Schaich, Plenum Press, New York, 1982, pp. 369-396.

9. Austin, Ann, private communication.